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Bioaccumulation and Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Related Compounds in Aquatic Ecosystems

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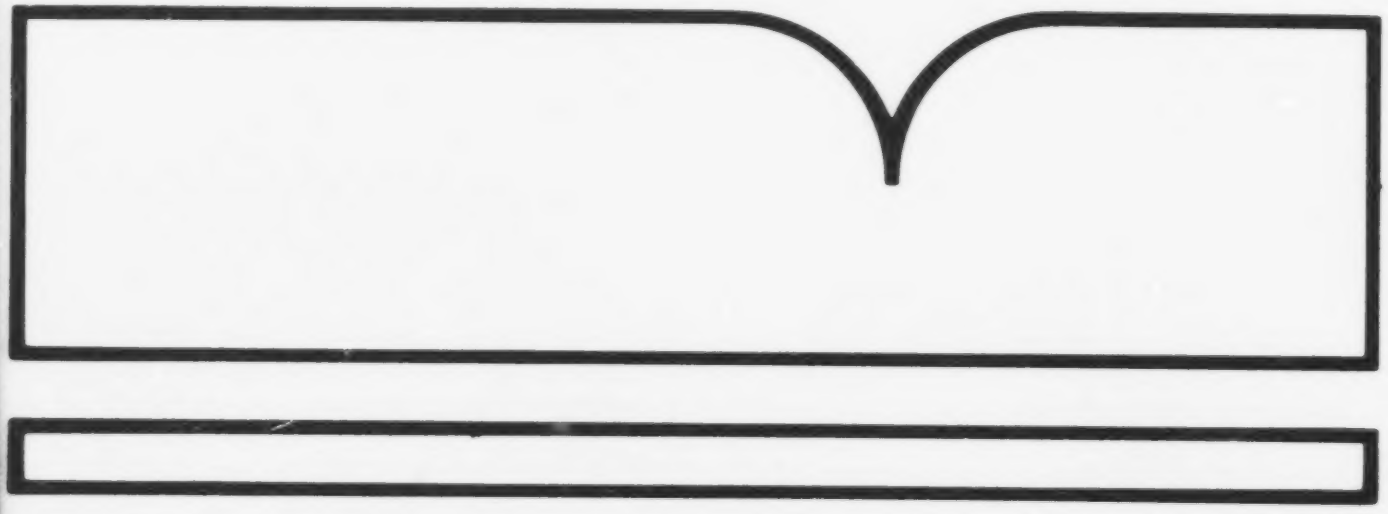

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Bioaccumulation and Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Related Compounds in Aquatic Ecosystems

by

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Running Head: TCDD Bioaccumulation and Toxicity in Aquatic Ecosystems

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SUMMARY

In order to complete ecological or human health risk assessments, exposure of aquatic organisms to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related chemicals must be linked to toxic or other biological effects in fish, wildlife or man. Two key elements of this linkage are: (1) the bioconcentration factor (BCF) of TCDD, and (2) the identification of the most sensitive biological effects associated with TCDD exposure.

Mean steady-state BCFs determined in the laboratory for three groups of young carp and two groups of fathead minnows exposed to TCDD in constant water flow systems were 66,000 and 128,000, respectively. On the basis of TCDD concentration in fish lipid, BCF_ℓ values for young carp, fathead minnows and rainbow trout are all approximately 730,000. This means that the BCF for fish with 7% lipid, under these exposure conditions, would be 51,000. The amount of TCDD in carp and rainbow trout tissues in the bioconcentration studies that is associated with 50% lethality is approximately 1000-2000 pg TCDD/g fish, a level only 6-12 times greater than that of the most contaminated fish found recently in Lake Ontario.

TCDD and related chemicals in Great Lakes fish are not known to cause overt toxicity in adults; however, early life stages of those freshwater fish species studied are more sensitive than adults. In order to assess the sensitivity during early development and to identify biological effects associated with TCDD exposure, fertilized lake trout eggs, obtained from a naturally reproducing population of feral Lake Superior lake trout, were exposed for 48 hours to waterborne TCDD. Hatchability was reduced at egg TCDD concentrations $\geq$ 226 pg/g. At lower whole egg TCDD concentrations the greatest mortality occurred during the sac fry stage. Prior to death, TCDD-exposed sac fry developed severe subcutaneous yolk sac edema and hemorrhages, morphologically resembling blue-sac disease. The no observable adverse effect level (NOAEL) for sac fry mortality was 34 pg TCDD/g and the lowest observable adverse effect level (LOAEL) was 55 pg TCDD/g, based on TCDD concentrations in the egg. The egg TCDD concentration that caused 50% mortality above control

at swim-up (LD50) was 65 pg TCDD/g. Whether early life stage development of lake trout will be similarly affected by other toxic polychlorinated dibenzo-p-dioxin (PCDD), dibenzofuran (PCDF) and biphenyl (PCB) congeners remains to be determined. The prediction of when or where trout eggs may exceed the LOAEL for sac fry mortality requires bioaccumulation factors that may be obtained from bioaccumulation models that incorporate bioconcentration potential, bioavailability conditions, food chain type, ecosystem characteristics and chemical hydrophobicity.

INTRODUCTION

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[1], and other planar polyhalogenated aromatic hydrocarbons share an ability to induce cytochrome P-4501A1 and exert a range of toxic effects in different species through an Ah receptor-mediated mechanism of action. An important consideration in human health and ecological risk assessments for these chemicals is their presence in aquatic ecosystems. Aquatic food chains often contribute significantly to human and wildlife dietary PCDD, PCDF and PCB exposures. Also, species associated with aquatic food webs, especially fish and fish-eating birds and mammals, appear to be very sensitive to polyhalogenated aromatic hydrocarbon toxicity. Thus the potential exists, at existing levels of environmental contamination, for those PCDD, PCDF and PCB congeners that act through the Ah receptor to elicit (in combination) adverse effects on certain species of fish and wildlife.

Since TCDD is the most toxic halogenated aromatic chemical known, it has been used as the reference chemical for the development of toxic equivalency factors (TEFs) (Safe 1990) and it has been the focus of state, national and international environmental regulations and standards. We have in recent years conducted studies of its bioavailability, bioaccumulation and toxicity to freshwater fish. Results of these studies, including previously unpublished work, are presented here to illustrate the exposure and hazard assessment components of an aquatic ecological assessment for TCDD and related chemicals.

Exposure and Bioavailability to Fish

The presence of TCDD and related PCDDs, PCDFs and PCBs in aquatic biota has been well documented (Stalling et al. 1983; Kuehl et al. 1987; Kuehl et al. 1989; Zacharewski et al. 1989). These chemicals share a high degree of hydrophobicity which increases as the degree of chlorination increases. This corresponds in general with an increase in lipophilicity and an increase in ability to bind to organic carbon in sediments and to dissolved organic matter in water (Servos and Muir 1989). The increase in lipophilicity associated with increasing degree of chlorination is not paralleled by an increase in bioaccumulation, however. Reduced bioavailability[3], due to sorption to organic matter and metabolic transformation of many PCDD and PCDF congeners by fish to polar metabolites that are eliminated, results in a much less bioaccumulation than expected on the basis of octanol-water partition coefficients (K_{ow}) (Muir et al. 1986; Gobas 1990). Molecular sizes and steric factors may also play a role in reducing accumulation of highly chlorinated congeners (Opperhuizen et al. 1985).

Risk assessments for point or non-point sources of these chemicals to aquatic ecosystems, are complicated by a broad range of factors that influence exposures to fish and other aquatic organisms. Transport and fate of the chemicals in the ecosystem governs the quantity of chemical available for contact with an aquatic organism. Mass balance models (DiToro et al. 1987) can be used to predict amounts of chemical present in an organism's exposure environment. Bioavailability (Fig. 1) of hydrophobic organic chemicals is influenced by the partitioning of the chemical in the water, sediment and food chain that a particular organism such as a lake trout contacts over an extended period. As previously mentioned, the amount of organic carbon, both in sediments and suspended in the water column, will influence the environmental distribution of the chemical and its relative availability for incorporation into the food chain, for bioconcentration through gill or dermal uptake or for assimilation from ingested sediment. Accumulated chemical, in the form of a tissue residue in fish, is a key bioindicator of the potential

for biological effects in the fish and of the potential for unacceptable human or wildlife exposure through food. Although both sediment and water almost always contribute to overall bioaccumulation by pelagic organisms, the relative contribution of sediment can vary greatly due to contaminant loading history, ecosystem characteristics, food chain structure and hydrodynamic conditions.

TCDD and frequently other planar polyhalogenated aromatic hydrocarbons have not been detected in water from aquatic ecosystems even when biota are highly contaminated. Because surface sediments are a good indicator of the relative amount of chemical in the system over the time scale involved for bioaccumulation of superhydrophobic chemicals, we have employed the bioavailability index (BI) as a convenient measure of site-specific bioaccumulation potential (Kuehl et al. 1987; Cook et al. 1990):

$$BI = C_{lipid}/C_{oc} \quad (1)$$

where C_{lipid} = mass of chemical/mass of lipid in the organism and C_{oc} = mass of chemical/mass of organic carbon in the surface sediment to be used as a reference for exposure. The BI consistently demonstrates an extremely wide range in bioavailability for PCDDs and PCDFs. Only 2,3,7,8- chlorine-substituted congeners are bioaccumulated by fish although much larger quantities of other PCDD and PCDF congeners are found in sediments. Metabolism of PCDDs and PCDFs in fish is an important mechanism for creating this pattern (Muir et al. 1986; Gobas 1990). Higher chlorinated 2,3,7,8-substituted congeners are very slowly accumulated but also have very slow elimination rates and therefore must be either slowly metabolized or not metabolized. This pattern of bioaccumulation should be considered when applying a TCDD toxicity equivalent (TEQ) approach (Barnes et al. 1986). The TEQ calculation will change significantly depending on whether the chemical mixture is measured at its source, in the food chain or in fish, wildlife or man. We have normally found that total TEFs for PCDDs and PCDFs in fish are dominated by the TCDD TEF contribution.

Bioaccumulation of TCDD by Fish

The bioaccumulation factor (BAF) is the ratio between the concentration of a chemical in an organism's tissues and the concentration of the chemical dissolved in the water in which the organism lives. The BAF is generally defined as the ratio that results at thermodynamic equilibrium from water, food and sediment routes of exposure. Bioaccumulation factors are difficult to measure for fish because exposure conditions that allow thermodynamic equilibrium are difficult to find or create for very hydrophobic chemicals and the concentration of chemical dissolved in water cannot be measured accurately. In reality, BAF measurements are often based on steady-state exposure conditions. Because bioaccumulation of hydrophobic organic chemicals is strongly influenced by the lipid content of the organism, a lipid-based BAF (BAF_ℓ) is preferred:

$$BAF_\ell = C_\ell / C_\omega \qquad (2)$$

where C_ℓ = mass chemical ÷ mass organism lipid and C_ω = mass dissolved chemical ÷ mass water.

When the combined accumulation from water and food produces a condition in which C_ℓ is greater than expected for equilibrium with C_ω, biomagnification has occurred (Thomann 1989). This can occur with a increase in fugacity of the food-borne chemical in the gastrointestinal tract when lipids are digested (Connolly and Pedersen 1988). Thus, in the absence of biomagnification, the equilibrium BAF equals the equilibrium bioconcentration factor (BCF) where bioconcentration refers to water exposure only with concomitant exchange of chemical across the gills. Since BCFs can be measured in controlled laboratory experiments (Veith et al. 1979), we have determined BCFs for four tetrachlorodibenzo-p-dioxins, including 2,3,7,8-TCDD, using the kinetic method (Branson et al. 1975). Unfortunately, the extreme hydrophobicity of these chemicals causes very significant underestimation of the uptake rate constant (and thus the BCF) when the chemical concentration in the exposure water is not adjusted for sorption to organic matter in the water (Gobas et al. 1989). Because the fraction of bioavailable chemical in BCF determinations is

uncertain, the BCF is usually calculated on the basis of the total chemical concentration in the exposure water. Consequently, BCF measurements for chemicals with octanol-water partition coefficients (K_{ow}) greater than 10^6 will greatly underestimate equilibrium BAFs for which C_w is based on dissolved chemical only.

In the absence of biomagnification or a significant increase in elimination rate due to metabolism, the maximum lipid-normalized bioconcentration factor is:

$$BCF_\ell = BAF_\ell = f_\ell . K_{ow} \qquad (3)$$

where f_ℓ = the fraction of lipid in the fish and K_{ow} is the octanol-water partition coefficient, a surrogate for lipophilicity which is estimated to be approximately 10^7 for tetrachlorodibenzo-p-dioxins (Burkhard and Kuehl 1986). Thus a fish with 7% lipid could achieve at equilibrium a TCDD residue equal to 700,000 times the concentration dissolved in the water. Using the K_{ow} - BCF (measured after 28 day exposures to fathead minnows) correlation of Veith et al. (1979), a BCF of 178,000 is estimated for TCDD. Measured TCDD BCFs reported prior to this study were 9,270 for rainbow trout (Branson et al. 1985) and 7,900 for fathead minnows (Adams et al. 1986). Both of these studies employed short-term static water exposures that produced slow rates of accumulation by the fish. A 28 day exposure of juvenile rainbow trout to TCDD under continuous-exposure, flowing water conditions provided a steady-state BCF estimate of 39,000 (Mehrle et al. 1988). To the best of our knowledge, the only other long-term continuous flow BCF determinations for TCDD are those we report here for carp and fathead minnows.

TCDD Toxicity in Juvenile and Adult Freshwater Fish

Freshwater fish, exposed to TCDD as juveniles or as adults with a single ip dose, are intermediate between the two most sensitive mammalian species, the guinea pig and the rat, in their sensitivity to TCDD-induced mortality. The LD50 of TCDD in adult yellow perch, carp, bullhead, rainbow trout, largemouth bass and bluegill ranges from 3,000-16,000 pg TCDD/g fish.

Mortality in TCDD-exposed fish is delayed in onset and can occur as long as 11 weeks after a single dose (Kleeman et al. 1988).

The gross signs of TCDD toxicity in fish, exposed as juveniles or adults, are delayed in onset and are species-dependent. Decreased growth rate and fin necrosis is observed in most species (yellow perch, carp, bullhead, largemouth bass, rainbow trout, bluegill, northern pike, guppy and coho salmon) whereas cutaneous hemorrhage (yellow perch, carp and bluegill), hyperpigmentation (carp and largemouth bass) and edema (yellow perch, rainbow trout, carp and northern pike) occur in fewer species. Whether the route of TCDD exposure is via the water, diet or by intra-peritoneal injection, or the duration of exposure is short or long, essentially the same signs of toxicity are produced within a given species of fish (Miller et al. 1979; Helder 1980, 1981; Cook et al. 1986; Spitsbergen et al. 1988a,b; Kleeman et al. 1988).

The histopathology in TCDD-exposed adult fish is characterized by epithelial, lymphomyeloid and cardiac lesions. The particular organs affected and the type, incidence and severity of the lesions are species-specific. In TCDD-exposed yellow perch and rainbow trout, epithelial lesions involve the liver, pancreas, gills and fins and lymphomyeloid lesions consist of thymic involution, splenic lymphoid depletion and hypocellularity of head kidney hematopoietic tissue. However, only in rainbow trout was an epithelial lesion of the gastric mucosa detected and only in yellow perch were hyperplasia of the gill filament epithelium, pericarditis and myocardial necrosis observed. The TCDD body burden in fish required to produce these morphologic lesions appears to be in the same range as that needed to produce gross signs of toxicity (Spitsbergen et al. 1988a,b).

Immunosuppression and decreased resistance to viral disease is observed in TCDD-exposed rainbow trout, but only at body burdens that cause overt toxicity (Spitsbergen et al. 1988c). Therefore, some of the most sensitive sublethal effects of TCDD in mammalian species, thymic involution and immunosuppression, are not produced by sublethal body burdens of TCDD in juvenile rainbow trout. Whether reproductive success of adult freshwater fish

species is adversely affected by chronic low level exposure to TCDD over multiple generations has yet to be assessed.

Background concentrations of TCDD in feral freshwater fish from inland waters of North America range from nondetectable levels to whole body concentrations as high as 21-130 pg/g in Lake Ontario lake trout (Lake Ontario TCDD Bioaccumulation Study, 1990). Signs of gross toxicity are generally not seen in wild adult freshwater fish populations in North America because of the low background levels of TCDD. Sublethal effects were recently associated with fish exposed to pulp mill effluents containing TCDD and other TCDD mode-of-author chemicals (Munkitrick, et al.; Hodson et al. 1990). The most sensitive sign of TCDD toxicity observed in a laboratory exposure of yearling rainbow trout was thrombocytopenia and leukopenia observed at a whole body TCDD concentration of 1,000 pg/g (Spitsbergen et al. 1988a). Carp, chronically exposed to TCDD via the water, exhibited ascites, subcutaneous hemorrhage and fin necrosis after accumulating a whole body TCDD concentration in excess of 1,000 pg/g (Cook et al. 1986). Rainbow trout swim-up fry, continuously exposed to TCDD via the water, showed adverse effects on survival, growth and behavior after accumulating a body burden of 990 pg/g (Mehrle et al. 1988).

TCDD in Eggs of Feral Lake Trout

Early life stages of fish are more sensitive to TCDD toxicity than adults. Therefore, the potential impact of environmental levels of TCDD and related compounds on early life stages of fish probably poses the greatest risk to feral fish populations. The major route of PCDD, PCDF and PCB accumulation in eggs of wild female fish is not from the water column; rather it is from redistribution of these toxicants from maternal tissues to the egg yolk during vitellogenesis (Guiney et al. 1979; Niimi 1983; Vodicnik and Peterson 1985).

The concentration of TCDD in Lake Ontario lake trout eggs is approximately 17-33% of the TCDD concentration in the whole female lake trout from which the eggs were obtained (Cook and Lodge, unpublished results).

Accordingly, whole body TCDD concentrations of 21-130 pg/g in Lake Ontario female lake trout (Short et al. 1990; Zacharewski et al. 1989) should give rise to whole egg TCDD concentrations of 4-43 pg/g. Mortality due to blue-sac disease, a naturally occurring edematous syndrome in hatchery-raised salmonids (Wolf 1969; Roberts and Shepherd 1986), has been documented to occur in Lake Ontario lake trout sac fry (Symula et al. 1990; Simonin 1990). We have sought to determine if a dose-related increase in blue-sac disease and sac fry mortality could be produced in Lake Superior lake trout eggs exposed to graded concentrations of TCDD in the laboratory. The combination of this TCDD residue-based hazard assessment with an assessment of TCDD exposures to fish, including the prediction of egg TCDD residue levels through use of bioaccumulation models, illustrates the essential components of an ecological risk assessment for TCDD.

METHODS AND RESULTS

TCDD Toxicity During Early Development of Lake Trout

Fertilized eggs were obtained from Lake Superior lake trout (Salvelinus namaycush). The background egg TCDD concentration was below the limit of detection (< 0.7 pg/g) and the only toxic PCDD or PCDF congener detected in the eggs was 2,3,7,8-TCDF at a concentration of 2.3 pg/g (Cook and Lodge, unpublished results). The background concentration of TCDD-equivalents (TEQs) due to toxic PCB congeners in the eggs (i.e., coplanar and monoortho analogs of the coplanar PCBs) was estimated to be 41 pg/g. The TEQ concentration was calculated from the concentration of individual toxic PCB congeners in the eggs and the toxic equivalency factor (TEF) assigned to each congener based on its enzyme induction potency in rat hepatoma H4IIE cells relative to TCDD (T. Schwartz, U.S. Fish and Wildlife, Columbia, MO, unpublished results). When calculated with more recent TEFs for PCBs (Safe 1990), a whole egg TEQ of 15 pg/g was obtained.[2]

The freshly fertilized eggs, exposed to vehicle or graded concentrations of waterborne [^{3}H]-TCDD for 48 hr, accumulated 0 (control), 34, 55, 121, 226 or 302 pg/g (1,300 eggs/exposure group; 4 exposure groups/dose). Following

exposure the eggs were transferred to TCDD-free water and evaluated during early development for TCDD metabolism, elimination and toxicity (Walker et al. 1991). All radioactive TCDD residues extracted from eggs and sac fry were due to the parent compound; no metabolites were detected. [^{3}H]-TCDD was not eliminated from eggs or sac fry, but was eliminated from fry ($t_{1/2}$, 35-37 days).

Embryo development and survival did not appear to be affected by TCDD until approximately one week prior to the onset of hatching when toxicity was detected in embryos having the highest egg TCDD concentration. Although hatchability was reduced by whole egg TCDD concentrations $\geq$ 226 pg/g, mortality occurred at lower egg TCDD concentrations during the sac fry stage. Based on egg [^{3}H]-TCDD concentrations, resulting from a 48 hour exposure, the no observable adverse effect level (NOAEL) for mortality determined at the end of the sac fry stage of development was 34 pg/g; the lowest observable adverse effect level (LOAEL) was 55 pg/g; and the egg TCDD concentration that caused 50% mortality above control (LD_{50}) was 65 pg/g (Fig. 2).

All TCDD-exposed sac fry that died developed subcutaneous edema of the yolk sac and subcutaneous hemorrhages prior to death (Fig. 3). The yolk sac edema fluid was an ultrafiltrate of blood (Guiney et al. 1990) and its insidious formation was associated with a gradual cessation of blood flow to the yolk sac and body. This syndrome was found to be essentially identical to blue-sac disease (Spitsbergen et al. 1991) and to be the cause of death of the TCDD-exposed sac fry (Walker et al. 1991).

TCDD-induced cardiac and/or vascular dysfunction appear to cause the edema syndrome (Wisk and Cooper 1990; Spitsbergen et al. 1991). Since necrosis of capillary endothelial cells does not occur in lake trout sac fry exposed as eggs to TCDD (Guiney and Peterson, unpublished results) an increase in vascular permeability, without damage to the endothelium, is a plausible alternative mechanism of edema formation in this fish species (Theobald et al. 1983; Katz et al. 1984).

Bioconcentration of TCDD by Carp and Fathead Minnows

Because of the very long water exposure time required for fish to bioconcentrate TCDD to a steady-state residue level, residue data from fish collected periodically during a 71 day water exposure period and a 61 day depuration period (clean water) were used to determine uptake (k_1) and elimination (k_2) rate constants for first order kinetics (Hamelink 1977). The steady-state BCF = k_1/k_2. Five groups of 30 one-year-old carp (Cyprinus carpio) with an average weight of 15g/fish were placed into 40 liter glass aquaria (3 exposure, 2 control). One of these groups (tank T3B) had been previously exposed for 105 days to 4.8 ng/ℓ of a mixture containing 1,2,3,4-; 1,3,6,8- and 1,3,7,9-TCDD. Three groups of 90 fathead minnows (Pimephales promelas) with an average weight of 1g/fish were placed into 40 liter glass aquaria (2 exposure, 1 control). Water concentrations of TCDD were maintained with an automated electronic injector system that delivered a dimethylformamide (DMF) solution of TCDD at a rate of 22 pg TCDD/min. to a mixing chamber with 5 μm filtered Lake Superior water flowing into the aquaria at a rate of 111 mℓ/min. (four complete aquarium water volume changes/day). Control aquaria received DMF without TCDD. The nominal water exposure TCDD concentration of 200 pg/ℓ was reduced throughout the exposure period to approximately 60 pg/ℓ for all carp exposures and 49 pg/ℓ or 67 pg/ℓ for the two fathead minnow exposures. Sorption to organic material and bioconcentration by fish were responsible for the reduction in exposure water TCDD concentrations. All water leaving the TCDD exposure system was pumped through a series of prefilters and activated charcoal filters to remove TCDD prior to discharge (Batterman et al. 1989). The fish were fed commercial fish food pellets (< 0.5 pg TCDD/g) and water temperature was maintained at 25°C throughout the study. Following TCDD exposure, fish were removed to aquaria containing clean water for the depuration period.

Fish were sampled for residue analysis on days 0, 1, 15, 30, 45, 60 and 71 of the exposure period. The exposure was stopped on day 71 when signs of TCDD toxicity developed, particularly for carp. Due to TCDD-induced

lethality, fish were sampled only on days 30 and 61 of the depuration period. At least seven water samples were collected for TCDD analysis from each aquarium during the exposure period. Water and fish samples were analyzed for 2,3,7,8-TCDD with a Finnigan-MAT 4500 Quadrupole and a Finnigan-MAT 8230 double focusing GC/MS system using both 30 m DB-5 and 60 m SP-2230 chromatography columns. Sample soxhlet extraction, cleanup, including carbon/silica gel column chromatography, and the stable isotope labeled TCDD internal standard method for quantification has been previously reported (Kuehl et al. 1987). The BIOFAC Program (Blau et al. 1975) was used for fitting the time-dependent fish residue data assuming a first order uptake and elimination rate equation.

The concentrations of TCDD in water used for BIOFAC calculations are averages of measurements for seven samples collected from each aquarium throughout the 71 day exposure period. Although TCDD concentrations in water fluctuated due to injector system variability, fish removal for residue analysis did not appear to influence the mean TCDD concentrations which were within ± 15% of the TCDD concentrations measured for exposure water at the end of the exposure period. We recommend that replicate water samples, composited over the entire exposure period, and TCDD saturated water (low organic carbon concentration without a solvent carrier) from a generator column (Lodge 1989) be used for future determinations of BCF for superhydrophobic chemicals. The presence of even small amounts of solvent carrier in the exposure water increases the activity of the chemical in water and appears thereby to reduce the BCF.

All of the TCDD residue data for carp are plotted in Figure 4. TCDD residues were not corrected for fish growth because of individual fish variability and general wasting of many carp due to TCDD toxicity especially after the exposure period. Separate BIOFAC fits of each exposure system's data demonstrate excellent agreement. There was no apparent effect of prior exposure to the non-2,3,7,8-TCDD mixture of tetrachlorodibenzo-p-dioxins for tank 3B fish. The fathead minnow results were similar except that different

mean TCDD concentrations in water occurred, perhaps due to a larger fish size distribution, and thus biomass, in tank T2 which also had the lower TCDD concentration in water. The calculated kinetic data and steady-state BCFs, including lipid-normalized BCF_{ℓ}s, are presented in Table 1.

Both carp and fathead minnows exhibited a variety of toxic signs following exposure to TCDD. Although containing lower whole body TCDD concentrations (2200 pg/g), carp were more severely affected and eleven died just before or during the depuration period while none died in the control aquaria. Without removal of most fish for residue analysis, the number of deaths probably would have been much greater. Most carp exposed to TCDD displayed some combination of the following signs of overt toxicity: fin darkening, fin erosion, caudal fin deformation, hemorrhages in the lateral body wall, lateral line lesions, cranial deformation, edema, difficulty swimming, body wall ulcers, and exopthalmia. Although carp continued to feed, there was a general tendency for fat loss. Histological analyses of liver, spleen, gill, fins and gastrointestinal tract revealed extensive pathology (Johnson et al. 1991).

DISCUSSION

TCDD Toxicity in Fish

TCDD residues in adult fish from contaminated ecosystems have been found to exceed 100 pg/g without consideration of additional TCDD toxicity equivalents due to the presence of other planar polyhalogenated aromatic hydrocarbons. Rainbow trout fry and young carp, with whole body TCDD concentrations of approximately 1000 pg/g (Mehrle et al. 1988) and 2200 pg/g (this work) respectively, experienced significant mortality as a consequence of accumulating TCDD from water in bioconcentration experiments. Despite the low TCDD body burdens associated with mortality in these two fish species (1000-2000 pg/g), that indicate a sensitivity to TCDD approaching that of the guinea pig (Poland and Knutson 1982), occurrences of fish population declines attributable to lethality of adult fish in the environment have not been reported to our knowledge. Early life stages of fish are more susceptible to

overt TCDD toxicity than adults. The concentration of TCDD in eggs that caused a 50% increase in sac fry mortality over that of control eggs was 65 pg/g egg for lake trout and 300 pg/g for rainbow trout. TCDD toxicity occurring during early development was characterized by yolk sac edema and hemorrhages, morphologically resembling blue-sac disease (Walker et al. 1991).

Early life stage mortality due to blue-sac disease was reported to occur for fertilized eggs collected from Lake Ontario lake trout from 1977 until 1983 when egg collections stopped (Simonin 1990). We found that an essentially identical syndrome could be produced in Lake Superior lake trout sac fry by egg TCDD concentrations as low as 40 pg/g (Spitsbergen et al. 1991) and 55 pg/g (Walker et al. 1991) and a syndrome consisting of edema and multifocal hemorrhages is also caused by TCDD during early development in pike, rainbow trout and medaka (Helder 1980, 1981, 1982; Wisk and Cooper, 1990). The significance of these findings for aquatic ecosystems is that they raise the possibility of high background levels of PCDDs, PCDFs and PCBs in feral fish eggs interacting to produce early life stage mortality (Walker et al. 1991). The environmental impact of such an effect would be a reduction in the population of the affected fish species in those inland waters where exposure of that species to halogenated aromatic hydrocarbons is occurring.

TCDD and related compounds are potent developmental toxicants in mammalian, avian and fish species and recent epidemiological studies suggest that humans exposed in utero to these chemicals are at greater risk of toxicity than those exposed as adults (Rogan et al. 1988; Rogan and Miller 1989). The biological basis for the greater susceptibility of early life stages to halogenated aromatic hydrocarbon toxicity is unknown. Also it is not known to what extent developmental effects of TCDD in humans are secondary to indirect maternal effects as opposed to direct effects on the embryo and fetus. Research in mammalian species cannot entirely resolve this latter issue, but studies in avian and fish species, where embryonic development

occurs outside of the mother's body, might shed light on the direct effects of these persistent chemicals on the developing organism.

Bioaccumulation of TCDD by Fish

A summary of all 2,3,7,8-TCDD BCF determinations for fish (Table 2) indicates a range of 7,900 to 159,000 for fathead minnows, yet the elimination rate constants (k_2) differ by only a factor of 3.7. The disparity lies in the estimate of the uptake rate constants (k_1). This was likely due to reduced bioavailability of TCDD associated with organic carbon build-up and limited mass of TCDD added in static exposure systems. The available TCDD was removed through bioconcentration by the fish leaving only TCDD that was primarily sorbed to organic matter in the water. The results suggest that equilibrium between fish, sorbents and water was not reached due to a desorption rate slower than the rate of gill uptake. Static renewal exposures would be characterized by rapidly declining TCDD uptake between periodic additions of TCDD. The exposure level reported for rainbow trout (Branson et al. 1985) is 27 times greater than the solubility of TCDD in water at 10°C (Lodge 1989). Although the water concentration used for BCF determination was reduced to 107,000 pg/ℓ to adjust for bioavailability, it is likely that the actual fraction of TCDD available for bioconcentration during the six hour static exposure was only a small fraction of this value.

Fish size, age, and lipid content also influence bioconcentration kinetics. Steady-state lipid normalized BCFs ($BCF_ℓ$) for the long-term flow through exposures of rainbow trout (Mehrle et al. 1988), carp and fathead minnows (this study) ranged from 510,000 to 830,000 (mean of 728,000). This means that a fish with 7% lipid should have a BCF of approximately 51,000. This number can underestimate an equilibrium BAF for TCDD since it is not calculated on the basis of a dissolved TCDD concentration and thus does not represent a true equilibrium constant. Through TCDD analysis of centrifuged exposure water we determined that at least 85% of the TCDD was bound to suspended solids. Estimates of k_1 for carp and fathead minnows on the basis of gill oxygen clearance (R. Erickson, personal communication) are

surprisingly close to the measured values. This suggests that most of the TCDD in the water was bioavailable. Unless the respiration rate is underestimated due to fish activity and/or TCDD effects on gill function, contradictory bioavailability estimates exist at this time.

The elimination rate constants (k_2) may be slightly increased by a slow rate of TCDD metabolism (Kleeman et al. 1986 a,b; 1988) but the steady-state BCF_ℓ estimates reported in Table 1 for this study are close to maximum BCF_ℓ values estimated with equation (2) for $K_{ow} = 10^7$ and $f_\ell = .09$ for carp and $f_\ell = .19$ for fathead minnows. If the actual K_{ow} for TCDD is greater, a much larger difference between the measured BCF_ℓs and equilibrium BAFs (no metabolism, no biomagnification) would exist.

We have measured TCDD elimination in adult carp with long-term environmental exposure through water, food and sediment (Kuehl et al. 1987). 1.5 kg carp with 16% lipid were collected from the Wisconsin River and maintained in Lake Superior water for 336 days. The $t_{1/2}$ for TCDD elimination was approximately 320 days with $k_2 = 0.0022$ days^{-1} in comparison to $t_{1/2} = 63$ days and $k_2 = 0.011$ days^{-1} for this study. This five-fold slower elimination rate may be explained simply on the basis of size and lipid differences for the two groups of carp.

In Lake Ontario we estimated, on the basis of a mass balance model-predicted water TCDD concentration of 0.25 pg/ℓ (Endicott et al. 1990), that a field BAF_ℓ for lake trout, with average TCDD residues of 35 pg/g and 18% lipid, is approximately 780,000 (BAF = 140,000) (Cook et al. 1990). This estimate is associated with great uncertainty for the TCDD concentration in Lake Ontario water and probably is smaller than the actual BAF_ℓ. It seems reasonable that the Lake Ontario BAF_ℓ would be larger than laboratory BCF_ℓ measurements due to accumulation of TCDD through the food chain route.

Risk Assessment of TCDD and Related Compounds in Aquatic Ecosystems

We are currently investigating the application of the lake trout sac fry response to egg contamination by TCDD (Walker et al. 1991) for ecological risk assessments of TCDD and related compounds in the Great Lakes. Caution must be

used when extrapolating these laboratory results to field conditions. In the environment the toxicity of TCDD and related chemicals during lake trout early development could be influenced by many factors including the bioavailability of the chemicals after maternal transfer, variation in water temperature, and genetic differences. A study in progress is designed to determine whether maternal transfer of TCDD to lake trout eggs provides the same relationship between egg TCDD concentration and early life stage mortality (due to a blue-sac syndrome) as when the eggs were exposed to waterborne TCDD (Walker et al. 1991; Spitsbergen et al. 1991). The assumption that TCDD and related chemicals act additively has never been unequivocally determined nor are other interactions well understood. Also, the presence of other contaminants could modulate the toxicity of these chemicals. Finally, TEFs based on mammalian responses to chlorinated dioxins, furans, and biphenyls relative to TCDD have never been compared to toxic responses of fish.

Models are required in order to connect sources of contaminants to exposures, residues and effects. Because bioaccumulation of hydrophobic organic chemicals is controlled by factors such as food chain structure, chemical properties and bioavailability at exposure, the estimation of field applicable BAFs becomes very site-specific. Thus the development of food chain models for application to specific bioaccumulation prediction problems continues among different research groups. Regulatory agencies would benefit from the development of site-specific BAFs for use when complete food chain model application is not feasible due to time or cost factors. We propose that an approach intermediate between use of a laboratory BCF measurement and application of a food chain model for site conditions may be advantageous.

Development of generic coupled benthic and pelagic food chain models for different types of aquatic ecosystems could provide a capability to select BAF_{ℓ}s based on ecosystem/food chain characteristics, trophic level, chemical hydrophobicity/lipophilicity and bioavailability conditions. Figure 5 provides an illustration of this framework. The choices of ecosystem types, such as oligotrophic, cold lake, etc., are for illustrative purposes only.

Ecologists, modelers, chemists, toxicologists etc., would need to plan a strategy for defining and developing a range of ecosystem-specific food chain model types. Even within an ecosystem type, food chain variations can result in measurable differences in bioaccumulation of PCBs (Rasmussen et al. 1990), and presumably other persistent lipophilic organic chemicals, by top trophic level species such as lake trout. The key to the generic BAF_ℓ model approach is to narrow ranges of BAF_ℓ uncertainty through stages of model development and application from general to successively more specific ecosystem structures and conditions. Resulting spreadsheets of BAF_ℓs would allow the user to select a BAF_ℓ for a particular application based on definition of the most appropriate food chain model, trophic level, K_{ow} range for the chemical of interest and site bioavailability condition (log C_{oc}/C_ω), where C_{oc} is the expected concentration of the chemical in surface sediment organic carbon and C_ω is the expected average chemical concentration in overlying water. C_ω should probably be normalized for suspended organic carbon in the water (DiToro 1985; Clark et al. 1990).

We have attempted to provide essential elements of an ecological risk assessment for TCDD and related compounds in aquatic environments. Exposure assessment includes source to receptor transport of chemical, bioavailability of the chemical through multiple exposure routes and bioaccumulation potential. Our discussion of hazard assessment focused on possible reproductive impairment in fish. Until TCDD and related chemical residues in fish eggs are more predictable from knowledge of the female's body burden, risk assessors may want to monitor fish eggs in order to most accurately determine the potential for reproductive impairment. Other effects may emerge as more sensitive determinants of aquatic hazards. The risk characterization process must balance these hazards with wildlife and human health hazards associated with dietary exposure to fish and shellfish containing residues of these chemicals.

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FOOTNOTES

[1]We use the notation "TCDD" to refer exclusively to 2,3,7,8-tetrachloro-dibenzo-p-dioxin.

[2]We caution that TEFs used for PCB congeners (T. Schwartz personal communication; Safe 1990) in the TEQ calculations have not been validated for use in fish.

[3]We have chosen to use the term "bioavailability" in its broadest sense. Thus in this report it represents the fraction of total chemical in the animals' exposure environment (water, food, sediment) that can be bioaccumulated and distributed to organs that are sites for toxic effects. The chemicals' properties, exposure medium conditions and internal biochemical factors such as metabolism or protein binding contribute to overall bioavailability.

FIGURE LEGEND

Fig. 1. Bioavailability of hydrophobic organic chemicals for fish: Role of chemical form, route of exposure, food chain and toxicokinetics on residue formation and potential for biological effects.

Fig. 2. Cumulative mortality of lake trout at swim-up as a function of the TCDD concentration in the egg. Cumulative mortality in TCDD groups has been corrected for mean cumulative mortality in the acetone control group (20.4%). Cumulative mortality is expressed on the ordinate on an angular transformation scale. Each point is cumulative mortality for a single TCDD replicate. Adapted with permission from Walker et al. (1991).

Fig. 3. Representative lake trout sac fry exposed as a fertilized egg to vehicle (top panel) or to TCDD in vehicle (bottom panel). Signs of TCDD toxicity resemble those of blue sac disease in that both are characterized by yolk sac edema and subcutaneous hemorrhages. Adapted with permission from Spitsbergen et al. (1991).

Fig. 4. Bioconcentration of 2,3,7,8-TCDD: BIOFAC fit of residue data for uptake and elimination phases of three exposures of TCDD in water to carp.

Fig. 5. Framework for predicting bioaccumulation on the basis of ecosystem characteristics, food chain structure, chemical hydrophobicity and measured or estimated site bioavailability conditions for exposures.

Table 1. Steady-state Bioconcentration Factor (ss BCF) Calculations for 2,3,7,8-TCDD

TCDD Exposure Tank	T3A	T3B*	T5	All Carp	T2	T4
Species	Carp	Carp	Carp	Carp	Fathead	Fathead
C_w (pg TCDD/ml water)	0.057	0.061	0.067	0.062	0.049	0.067
Exposure period (d)	71	71	71	71	71	71
Depuration period (d)	61	61	61	61	61	61
k_1, uptake rate constant (ml/g.d)	736	765	712	720	1280	1870
k_2, elimination rate constant (1/d)	0.008	0.012	0.014	0.011	0.012	0.013
Elimination half-life, $t_{1/2}$ (d)	84	58	48	63	52	59
ss BCF (C_f/C_w) x 10^{-3}	89 ± 25	64 ± 16	49 ± 7	66 ± 9	97 ± 5	159 ± 40
ss BCF_ℓ (C_ℓ/C_w) x 10^{-4}	162 ± 158	75 ± 20	51 ± 5	73 ± 14	51 ± 3	84 ± 21

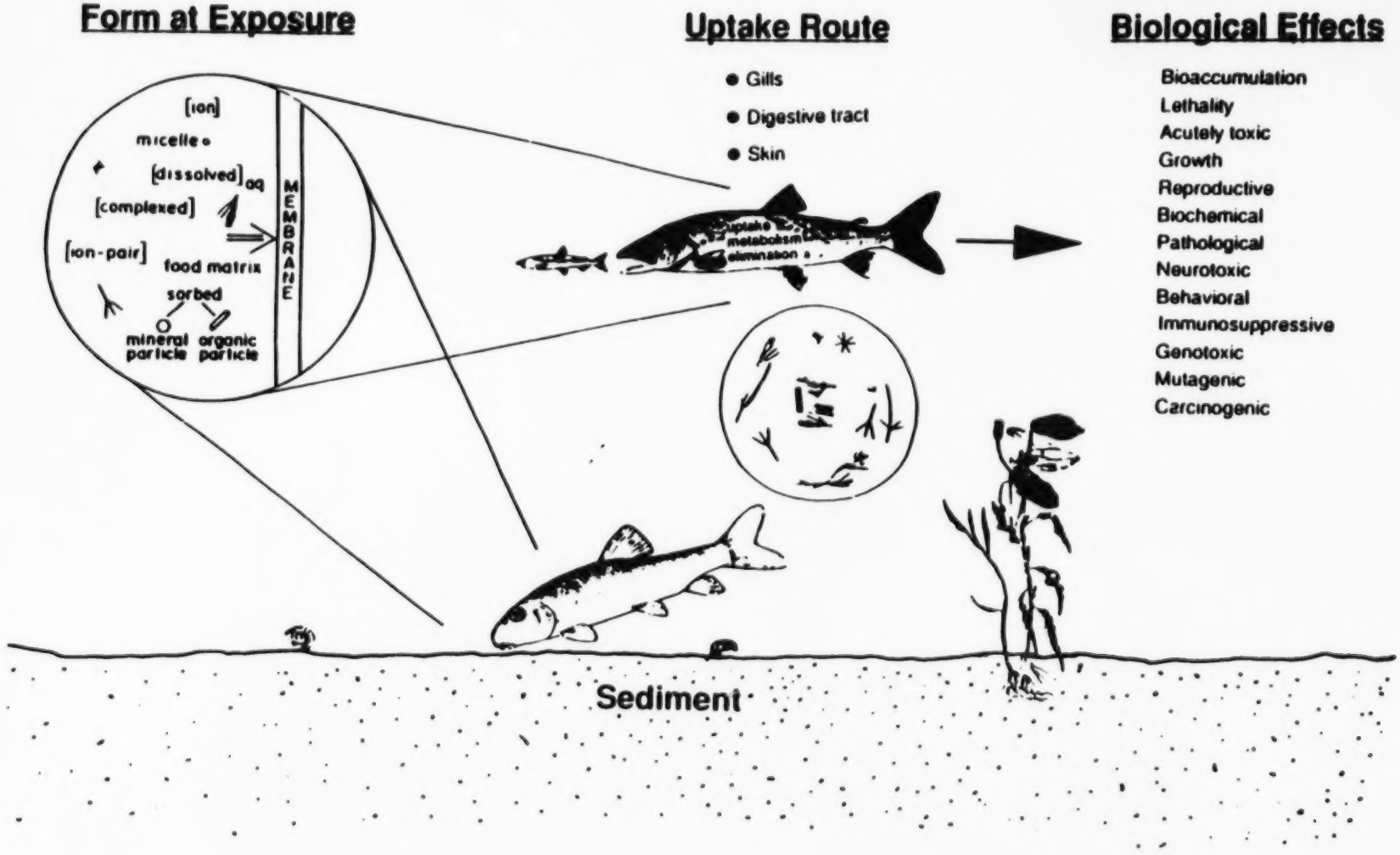
*T3B carp were previously exposed for 105 days to a mixture of 1,2,3,4-; 1,3,6,8-; and 1,3,7,9-TCDD in water.

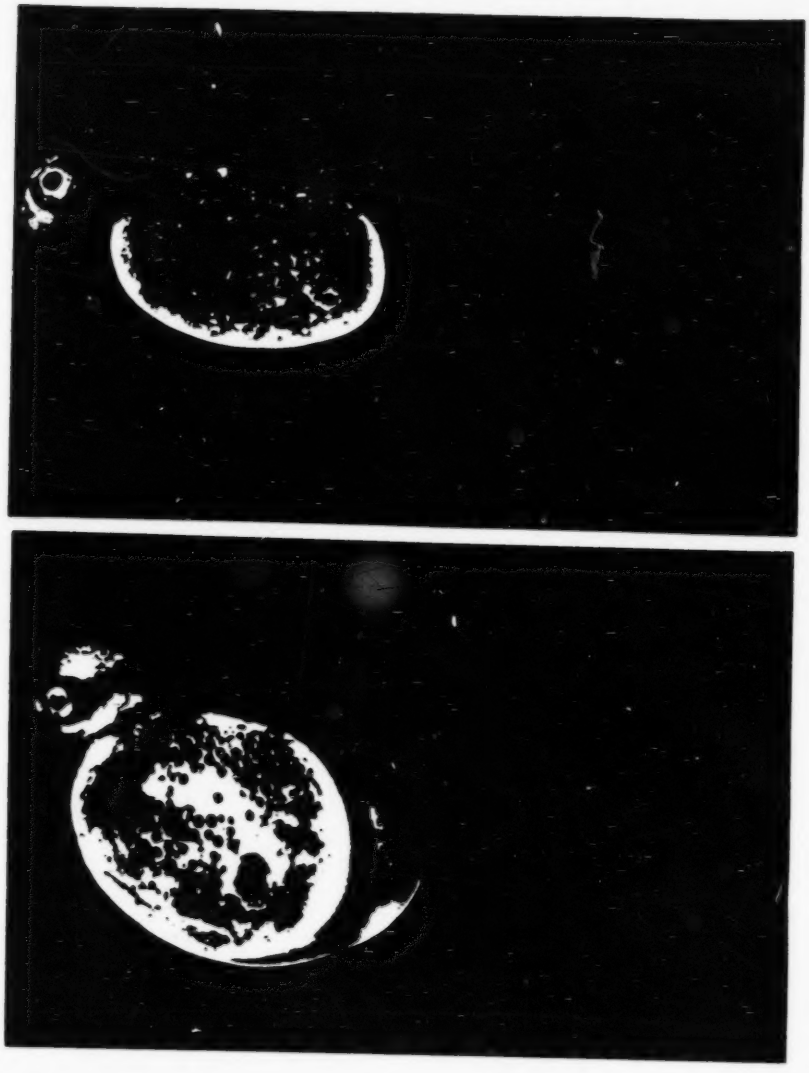
Table 2. Summary of 2,3,7,8-TCDD Steady-state Bioconcentration Factor (ss BCF) Determinations for Fish

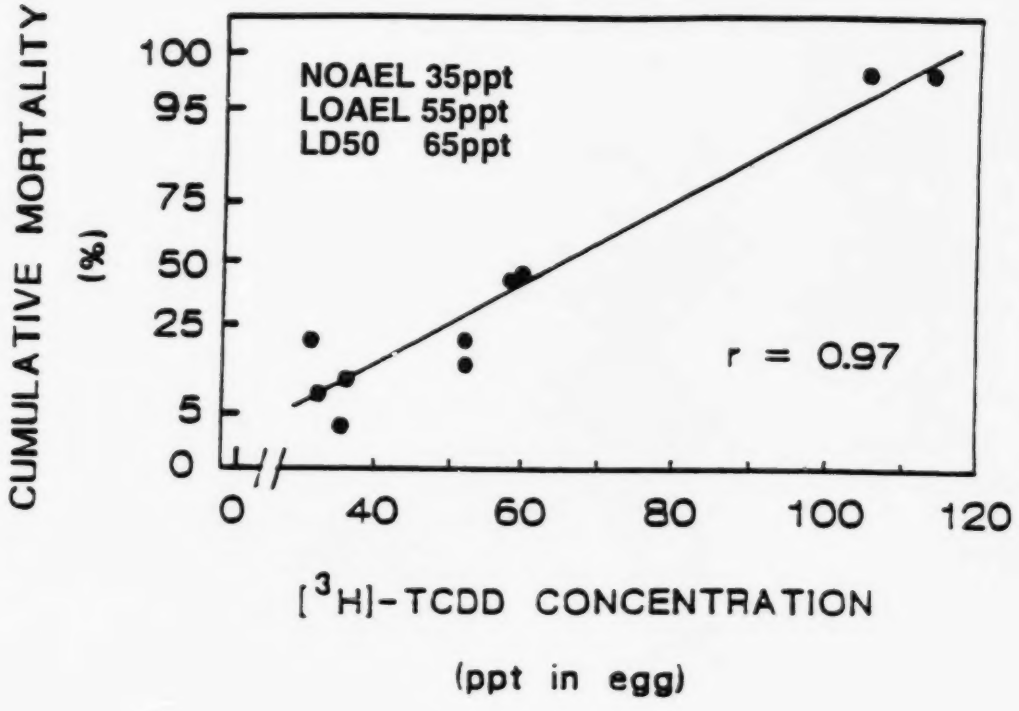
Study	Species	Initial Size(g)	% Lipid*	Temp. (°C)	TCDD Conc.(pg/l)	Exposure Period (days)	Depuration Period (days)	Uptake k_1	Elim. k_2	ss BCF	ss BCF_ℓ*
Branson et al. 1985	Rainbow trout	35	NR-est. 8	10	320,000	0.25-static	139	108	0.012	9,270	116,000
Mehrle et al. 1988	Rainbow trout	0.38 fry	NR-est. 5	11	38	28-flow through	none	1852	0.047	39,000	780,000
Adams et al. 1986	Fathead minnow	0.5-1.0 juvenile	NR-est. 7	25	1,000	28-static renewal	20	381	0.048	7,900	113,000
Cook et al. 1986, this report	Fathead minnow	1.0 young adult	19	25	49-67	71-flow through	61	1280-1870	0.012-0.013	97,000-159,000	510,000 837,000
Cook et al. 1986, this report	Carp	15	9	25	62	71-flow through	61	700	0.010	66,000	733,000

*Where not reported (NR), % lipid is estimated on basis of fish species, size and age.

Bioavailability

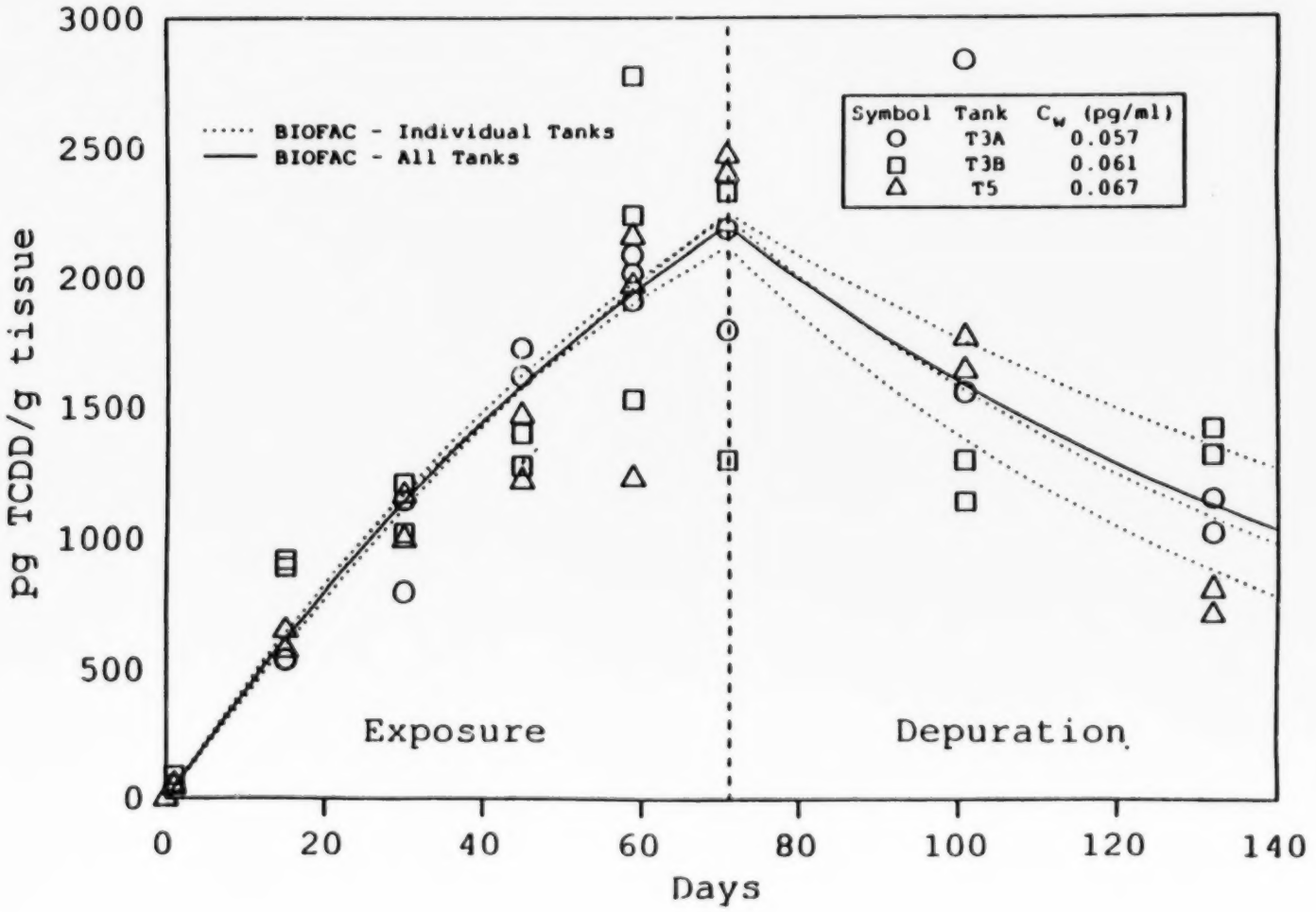


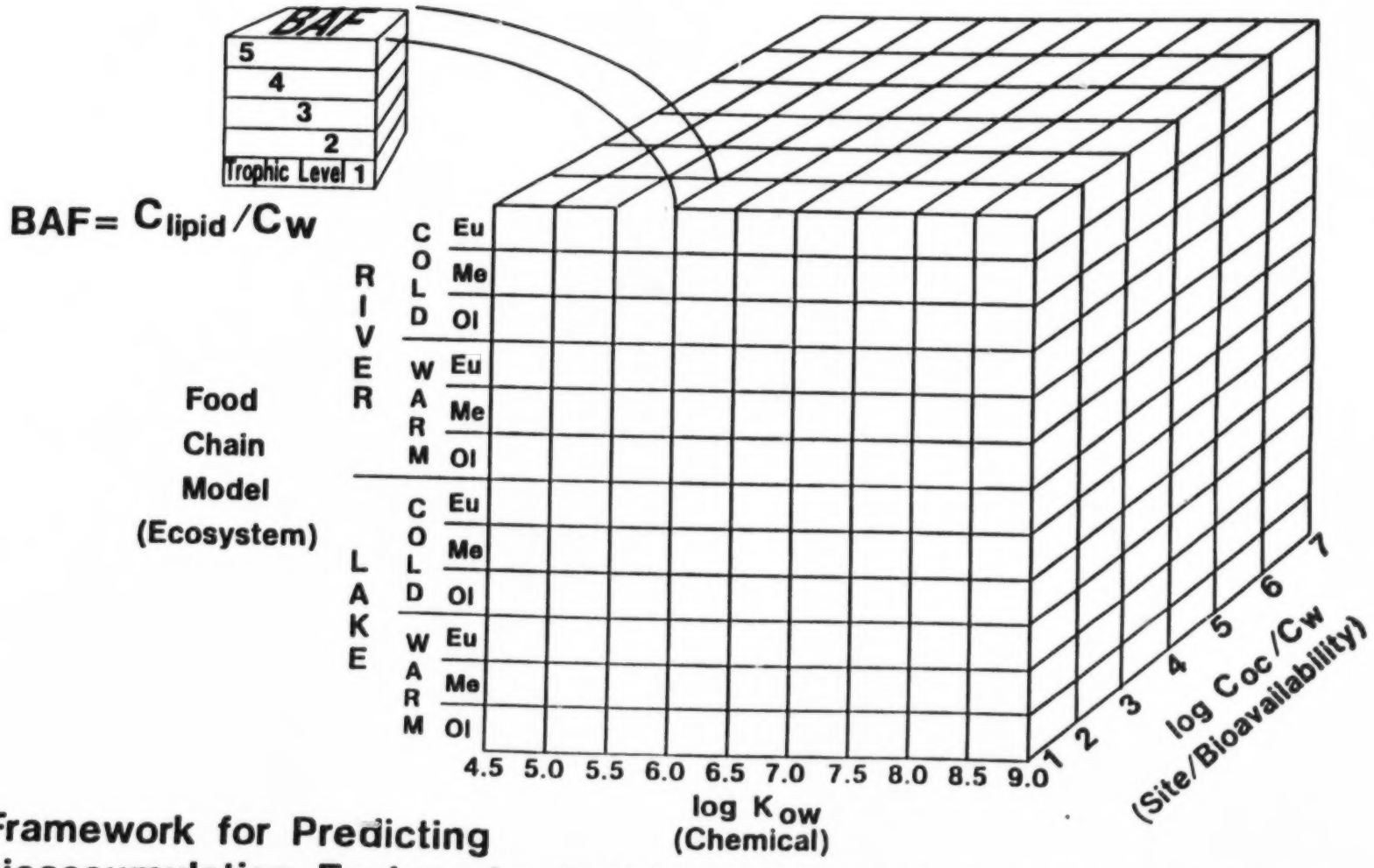



CUMULATIVE MORTALITY
(%)
100
95
75
50
25
5
0
NOAEL 35ppt
LOAEL 55ppt
LD50 65ppt
r = 0.97
0
40
60
80
100
120
[3H]-TCDD CONCENTRATION
(ppt in egg)

2378-TCDD Bioconcentration: Carp

All Data; Avg. C_w = 0.062 pg TCDD/ml water





Framework for Predicting Bioaccumulation Factors Applicable to Different Aquatic Ecosystems